Determining Luciferin Kinetic Curve for Your Model

Attached is a description of how to prepare and inject Luciferin. Investigators should determine for their model the optimal time after Luciferin injection to image, since biodistribution is rapid but the kinetics may be tissue dependent.

To generate a kinetic curve for luciferase activity in your model:

1. Inject Luciferin i.p. as described below. We prefer to inject into awake animals. If you need to sedate the mice before injection, that is fine, but it may slightly extend the kinetics (peak luciferase expression time).
2. Wait three minutes, then sedate by your method of choice, gas or injectable anesthesia.
3. Place sedated animals in imaging chamber and take the first image approximately five minutes after the Luciferin injection.
4. Continue to take images every 5-10 minutes up to about 40 minutes to generate a kinetic curve for Luciferin expression in your model. With 2% isoflurane gas anesthesia healthy animals can safely be sedated in the IVIS for 45 minutes.
5. Once you have established your curve you can choose the best time point to image at thereafter. We image most of our models at 10-20 minutes after Luciferin injection.

Imaging Procedure

Mice are injected by an intraperitoneal route with a Luciferin solution (15 mg/mL or 30 mg/kg, in PBS, dose of 150 mg/kg) that is allowed to distribute in awake animals for about 5-15 minutes. The mice are placed into a clear Plexiglas anesthesia box (2.5-3.5% isofluorane) that allows unimpeded visual monitoring of the animals; e.g. one can easily determine if the animals are breathing. The tube that supplies the anesthesia to the box is split so that the same concentration of anesthesia is plumbed to the anesthesia manifold located inside the imaging chamber. After the mice are fully anesthetized, they are transferred from the box to the nose cones attached to the manifold in the imaging chamber, the door is closed, and the "Acquire" button (part of the Living Image program) on the computer screen is activated. The imaging time is between one to five minutes per side (dorsal/ventral), depending on the experiment. When the mice are turned from dorsal to ventral (or vice versa), they can be visibly observed for any signs of distress or changes in vitality. The mice are again imaged (maximum five minutes), and the procedure is complete. The mice are returned to their cages where they awake quickly.

Preparation and Injection of Luciferin for *In Vivo* Bioluminescent Assays

Materials

- D-Luciferin, Firefly, potassium salt, 1.0 g/vial, DPBS, w/o Mg^{2+} and Ca^{2+}

Note: One can either reconstitute the entire 1.0 g of D Luciferin in 66.6 mL of DPBS or reconstitute the quantity of needed for an individual experiment.

- Syringe filter, 0.2 µM

Procedure

1. Prepare a fresh stock solution of Luciferin at 15 mg/mL or 30 mg/kg in DPBS. Filter sterilize through a 0.2 µM filter.
2. Inject 150 mg Luciferin/kg body weight. See protocol below. (e.g. For a 10 g mouse, inject 100 ul of 15 mg/ml to deliver 1.5 mg of Luciferin.)
3. Inject the Luciferin intra-peritoneally (i.p.) 5-15 minutes before imaging.

Note: A Luciferin kinetic study should be performed for each animal model to determine peak signal time after Luciferin administration.

Intraperitoneal (i.p.) Injection of Luciferin

Preferred Site

Animal's lower left abdominal quadrant.

Needle Size

25 gauge, usually used with 1 cc syringe

Volume

100 µL of Luciferin (15 mg/mL stock) per 10 grams of mouse body weight.

Note: 1 mL i.p. injection of a nonirritating solution is easily tolerated.

Position of Animal

Manually restrained, dorsal recumbency (abdomen side up), with cranial (head) end of animal pointed down.

Injection

Needle should be bevel-side up and slightly angled when entering the abdominal cavity. Penetrate just through abdominal wall (about 4-5 mm). The tip of the needle should just penetrate the abdominal wall of the animal's left lower abdominal quadrant.

上海乐辰生物科技有限公司

上海市宝山区共江路 299 号 207 室　200431

Tel: 021-60444836　400-0021-908　Fax: 021-66209682

Http://www.sinochrome.net　E-mail: tech@sinochrome.net

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